Biology

Cancer

c-Myc degradation

Deregulated expression of the proto-oncogene *c-Myc* has been implicated in malignant transformation of several human cancers. *c-Myc*-induced apoptosis, or growth while apoptosis is suppressed, can be achieved with low levels of *c-Myc* expressed at the wrong time in the cell cycle or for the wrong duration of time. This indicates that it is not merely the level of expression but the timing of events that is of major importance.

In a recent elegant study, Yeh. *et al.* deciphered the regulation of duration of c-Myc signaling at the molecular level [1]. Phosphorylation at Ser62 stabilizes c-Myc, whereas subsequent phosphorylation at Thr58 is required for its degradation via ubiquitination. Ser62 needs to be dephosphorylated by protein phosphatase 2A (PP2A) before ubiquitination of c-Myc can occur. PP2A prefers residues in which the adjacent peptidyl-prolyl bond is *trans*, which is ensured by Pin1 prolyl isomerase.

The identification of PP2 and Pin1 in the degradation of c-Myc is important because it links c-Myc pathways to pathways in which PP2A and Pin1 play major roles. PP2a is considered a tumour suppressor and the new role in c-Myc degradation corroborates this. However, Pin1 is thought to exert stimulatory effects on cell division via cyclins D1 and Cdc25. The relationship between these functions and the role of Pin1 in c-Myc degradation needs further investigation.

The authors demonstrate the functional relevance of their model because lack of Pin or PP2A leads to stabilization of c-Myc and full-blown transformation. Moreover, they shed light on the mechanism of action of SV40 small T antigen in transformation by demonstrating that it inhibits dephosphorylation, thus resulting in the stabilization of c-Myc.

1 Yeh, E. *et al.* (2004) A signaling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nat. Cell Biol.* 6, 308–318

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Targets and Mechanisms

A metabolite of chloroquine acts as a chloroquine-resistance reverser

Historically, chloroquine was used to treat malaria successfully, safely and cheaply. Today, however, it is largely ineffective as a result of almost universal resistance. Nonetheless, there remains a great deal of interest in its mechanism of resistance. The hope is that new compounds with similar properties, but that circumvent chloroquine resistance, can be developed.

Resistance is strongly linked to mutations in PfCRT, a protein located in the membrane of the food vacuole (FV) of *Plasmodium falciparum*. The FV is the probable site of action of chloroquine. The biochemical mechanism of resistance is still poorly understood, but appears to arise from outflow of the drug from the FV brought about by these mutations, lowering its local concentration. Several resistance-reversing agents are currently known, but generally these are too toxic for clinical use.

Now, Leann Tilley and coworkers [2] have made a startling observation. They have shown that 4-amino-7-chloroquinoline, a simple metabolite of chloroquine, is a potent chloroquine resistance-reverser. Thus, chloroquine can give rise to its own resistance-reverser upon metabolic breakdown. The authors suggest that if this metabolite reaches significant plasma concentrations it might, at least in part, explain the continued higher than expected efficacy of chloroquine in certain clinical settings. Perhaps most importantly, however, this simple and cheap compound could potentially be used in combination with chloroquine to restore its efficacy. Furthermore, it provides new insight into the relationship between molecular structure and chloroquine resistance as well as resistance-reversal.

2 Kalkanidis, M. *et al.* (2004) Synergistic interaction of a chloroquine metabolite with chloroquine against drug-resistant malaria parasites. *Biochem. Pharmacol.* 67, 1347–1353

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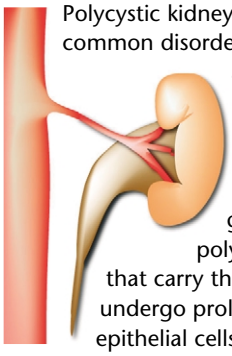
Vasopressin V2R inhibitors for curing polycystic kidney disease



Polycystic kidney disease (PKD) is a common disorder that affects around 1 in 500 people. It is transmitted through either of two autosomal genes that encode polycystins. Patients that carry these mutations undergo proliferation of renal epithelial cells, which also display excessive secretion, thereby forming cysts in kidneys, but also in liver, brain, pancreas and heart. The disorder is a major cause of life-threatening, end-stage renal disease, which affects both sexes at 40–60 years, and can not be treated at present except for last-resort kidney transplantation.

Recent molecular analysis has established that lack of either polycystin gene results in altered regulation of intracellular calcium and increased levels of cAMP. Vicente Torres *et al.* at the Mayo Clinic in Rochester (http://www.mayo.edu) asked themselves if blocking the Vasopressin V2 Receptor (VPV2R), a known cAMP agonist specifically expressed in kidneys, could be of therapeutic value for PKD [3]. Fortunately, the hypothesis could be tested because a selective inhibitor for this VPV2R receptor, OPC31260, developed by the Otsuka Pharmaceutical Company (http://www.otsuka.co.jp), is in Phase III clinical trials for patients that suffer from inappropriate antidiuretic hormone secretion.

The drug candidate was tested in an animal model for PKD, in mice that lack one of the polycystin orthologs. After four months of treatment with OPC31260, kidney size and cAMP levels were almost restored to normal. In addition to being hopeful news for PKD patients, a lesson from this work is that, like in the past for phosphodiesterase-5 inhibitors, target identification and validation once again appear to evolve with the development and availability of drugs, rather than merely being the initial event in an orderly, linear, 'rational' drug-discovery flowchart.

 PII: S1359-6446(04)03127-7

3 Torres, V. E. *et al.* (2004) Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat. Med.* 10, 363–364

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Proteomics and Genomics

High-throughput, RNAi-based target discovery for 15,000 genes

The introduction into cells of short, double-stranded fragments of RNA (siRNA or hairpin shRNA) can destroy endogenous mRNA that contains the same sequence. This discovery, made in 2001, is a special case of RNA-interference (RNAi) and many believe it has the potential to revolutionize the speed at which new drug targets can be discovered.

In a recent paper [4], scientists from Cold Spring Harbor Laboratory (http://www.cshl.org), The Baylor College of Medicine at Houston (http://www.bcm.tmc.edu) and Rosetta Inpharmatics (http://www/rii.com) have created a huge library of plasmids, each of which can express a defined siRNA against a known mRNA target. At present, the library consists of >37,000 sequence-verified plasmids that target human (>9,600) and mouse (>5,500) genes.

The team decided on vectors that each correspond to 29 nucelotides (nts) of target sequence, and included an extra 27 nts U6 promoter sequence for efficient transcription of the siRNAs. To add versatility, the vector inserts can be transferred, by efficient recombination, into other plasmid backbones that can produce a recombinant retro- or lentivirus, increasing the variety of cell assays on which the library can be tested. Finally, each vector has a 60 nts unique 'barcode' so that its presence can be detected in microarrays or by PCR in mixed cell populations. In a first proof-of-concept experiment, the team tested 4,900 genes, and 'scored' about 50% of the genes known to be involved in proteasome function.

The library is currently complete for all kinase and phosphatase genes, and will eventually encompass the entire genome. Clearly, the flexibility and the size of the library make this an unique research tool. Recently, there have been concerns over non-specific responses generated by the introduction into cells of double-stranded RNA, and other 'off-target' effects caused by sequence similarities between different genes. The choice for 29 nt-siRNAs would seem to favour efficacy over selectivity, and one might wonder if aiming at >3 mismatches with any other known transcript (a design criterion) is sufficient to ensure that only a single target will be hit in all cases. However, in this type of approach, casting a fine-mesh web makes sense, as 'positives' can (and must) be followed up with a set of control experiments.

4 Paddison, P.J. *et al.* (2004) A resource for large-scale RNA-interference-based screens in mammals. *Nature* 428, 427–431

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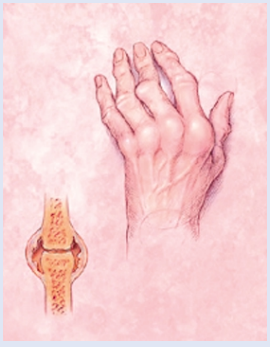
Immunology

A potential new role for MMPs in arthritis



Rheumatoid arthritis (RA) is considered to be an autoimmune disease involving several factors including the activation of autoreactive T-cells, release of pro-inflammatory cytokines and degradation of cartilage. Based on sequence similarity with the major histocompatibility complex (MHC)-binding residues of a peptide used to induce adjuvant arthritis *in vivo*, van Bilsen *et al.* [5] have identified possible self-antigens that could be responsible for the activation of autoreactive T-cells.

Subsequent screening of these self-antigen peptides demonstrated that several could activate the proliferation of cells isolated from various rat lymphoid organs *ex vivo*. Further studies confirmed that when applied to the hind paw of rats, a subset of these peptides could induce arthritis as determined by joint swelling and deformity.

However, not all peptides that induced *ex vivo* proliferation could induce arthritis *in vivo*, which suggests that the *ex vivo* studies are not fully predictive. Of the six peptides that were able to induce arthritis, three were derived from the matrix metalloproteinase family (MMP-3, -10, -16). These were shown to induce arthritis specifically via T-cell activation as confirmed in passive arthritis induction by T-cell transfer experiments, although the incidence rate was low. In addition, it was also shown that the a B-cell response to MMP-3 exists in experimental arthritis, as detected by the presence of IgG autoantibodies.

MMPs have previously been associated with regulating cytokines and the degradation of cartilage, but until now were not known to be of importance in the activation of autoreactive T-cells. Thus, modulation of MMP-induced T-cell autoactivation might offer a novel immunotherapy in the treatment of RA.

5 van Bilsen, J.H.M. *et al.* (2004) Matrix metalloproteinases as targets for the immune system during experimental arthritis. *J. Immunol.* 172, 5063–5068

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